

### **Amendment to the Claims**

#### **Claims**

1. (Original) A method of identifying a microRNA-recognition element comprising the steps of:

comparing degree of complementarity of a microRNA nucleotide sequence to an mRNA sequence to identify the presence of an mRNA sequence that exhibits a degree of complementarity to the microRNA sequence that is indicative of a microRNA-recognition element for the microRNA,

wherein the microRNA is 17-25 nucleotides and includes

a proximal region that is 7-9 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 5' end which is the microRNA's 5' terminus nucleotide,

a distal region that is 7-15 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 3' end which is the microRNA's 3' terminus nucleotide, and

a loop region that is 0 nucleotide, 2-3 nucleotides or 6-9 nucleotide,

wherein when the loop region is 0 nucleotides the 3' end of the proximal region is contiguous to the 5' end of the distal region and when the loop region is 2-3 or 6-9 nucleotides the 3' end of the proximal region is contiguous to the 5' end of the loop region and the 3' end of the loop region is contiguous to the 5' end of the distal region,

wherein complementarity of the mRNA sequence to the microRNA sequence that is indicative of a microRNA-response element for the microRNA is characterized by:

an mRNA sequence having a sequence that:

a) includes a region corresponding to the proximal region of the microRNA that is either completely complementary to the proximal region, or has a single mismatch to the 5' end of the proximal region, or symmetrically placed between the 5' end of the proximal region and the 3' end of the proximal region;

b) includes a region corresponding to the loop region of the microRNA that either forms a loop of 2-5 non-paired nucleotides when the loop region is 0, or has 0 nucleotides when the loop region is 6-9 nucleotides, or has 2-3 nucleotides which forms a

bulge of 2-3 non-complementary nucleotides of the loop region when the loop region is 2-3 nucleotides; and

c) includes a region corresponding to the distal region that is either completely complementary to at least 7 contiguous nucleotides of the distal region including the 5' end of the distal region, or contains mismatches of 1-4 contiguous nucleotides and matches of at least 5 nucleotides to a contiguous nucleotide sequence of the distal region including the 5' end of the distal region;

wherein an mRNA sequence that has complementarity to the microRNA sequence that is indicative of a microRNA-recognition element for the microRNA indicates that said mRNA sequence is a microRNA-recognition element for the microRNA.

2. (Original) The method of claim 1 further comprising determining free energy of microRNA bound to said mRNA sequence wherein a free energy determination of -10 kcal/mole or less indicates that said mRNA sequence is a microRNA-recognition element for the microRNA.

3. (Original) The method of claim 2 wherein a free energy determination of -20 kcal/mole or less indicates that said mRNA sequence is a microRNA-recognition element for the microRNA.

4. (Original) The method of claim 2 wherein a free energy determination of -30 kcal/mole or less indicates that said mRNA sequence is a microRNA-recognition element for the microRNA

5. (Currently Amended) The method of claim 1 ~~any of claims 1-4~~ wherein mRNA sequences in a database of mRNA sequences are compared to the miRNA sequence.

6. (Original) The method of claim 5 wherein repetitive Alu sequences are removed from mRNA sequences in a database of mRNA sequences and the remaining mRNA sequences are compared to the miRNA sequence.

7. (Currently Amended) The method of claim 1 ~~any of claims 1-6~~ wherein the miRNA sequence is compared to the mRNA 3' untranslated region.

8. (Currently Amended) A system for identifying a microRNA-recognition element comprising:

an input interface for inputting mRNA sequences, a database of mRNA sequences or a link for connecting to a remote data input interface, data or a database of mRNA sequences;

an input interface for inputting microRNA sequences, a database of microRNA sequences or a link for connecting to a remote data input interface, data or a database of microRNA sequences;

a processor with instructions for comparing mRNA sequences to microRNA sequences to identify a microRNA-recognition element according to the method of claim 1 ~~any of claims 1-7~~.

9. (Original) The system of claims 8 comprising a link for connecting to a database of mRNA sequences.

10. (Original) The system of claims 8 comprising an input interface for inputting microRNA sequences.

11. (Currently Amended) A computer program embodied on a computer readable medium for implementation on a computer system that for identifying a microRNA-recognition element, the program comprising instructions for performing the steps of the method of claim 1 ~~any of claims 1-7~~.

12. (Original) A method of generating a microRNA comprising the steps of:

identifying a selected mRNA sequence to be the target of the microRNA;

generating an oligonucleotide sequences that is 17-25 nucleotides and has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for a microRNA,

wherein the microRNA includes

a proximal region that is 7-9 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 5' end which is the microRNA's 5' terminus nucleotide,

a distal region that is 7-15 nucleotides, has a 5' end and a 3' end and includes a nucleotide at the 3' end which is the microRNA's 3' terminus nucleotide, and

a loop region that is 0 nucleotide, 2-3 nucleotides or 6-9 nucleotide,

wherein when the loop region is 0 nucleotides the 3' end of the proximal region is contiguous to the 5' end of the distal region and when the loop region is 2-3 or 6-9 nucleotides the 3' end of the proximal region is contiguous to the 5' end of the loop region and the 3' end of the loop region is contiguous to the 5' end of the distal region,

wherein complementarity of the mRNA sequence to the microRNA sequence that is indicative of a microRNA-response element for the microRNA is characterized by:

an mRNA sequence having a sequence that:

a) includes a region corresponding to the proximal region of the microRNA that is either completely complementary to the proximal region, or has a single mismatch to the 5' end of the proximal region, or symmetrically placed between the 5' end of the proximal region and the 3' end of the proximal region;

b) includes a region corresponding to the loop region of the microRNA that either forms a loop of 2-5 non-paired nucleotides when the loop region is 0, or has 0 nucleotides when the loop region is 6-9 nucleotides, or has 2-3 nucleotides which forms a bulge of 2-3 non-complementary nucleotides of the loop region when the loop region is 2-3 nucleotides; and

c) includes a region corresponding to the distal region that is either completely complementary to at least 7 contiguous nucleotides of the distal region including the 5' end of the distal region, or contains mismatches of 1-4 contiguous nucleotides and matches of at least 5 nucleotides to a contiguous nucleotide sequence of the distal region including the 5' end of the distal region;

wherein the oligonucleotide sequence has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA for a microRNA-recognition element.

13. (Original) The method of claim 12 further comprising the step of determining free energy of the microRNA bound to the selected mRNA sequence wherein a free energy

determination of -10 kcal/mole or less indicates that said mRNA sequence is a microRNA-recognition element for the microRNA.

14. (Original) The method of claim 12 wherein a free energy determination of -20 kcal/mole or less indicates that said mRNA sequence is a microRNA-recognition element for the microRNA.

15. (Original) The method of claim 12 wherein a free energy determination of -30 kcal/mole or less indicates that said mRNA sequence is a microRNA-recognition element for the microRNA

16. (Currently Amended) The method of ~~any of~~ claims 12-15 wherein the selected mRNA sequence is a known MRE.

17. (Currently Amended) The method of ~~any of~~ claims 12-15 wherein the selected mRNA sequence is in the 3' untranslated region of an mRNA.

18. (Currently Amended) The method of ~~any of~~ claims 12-17 wherein an oligonucleotide is synthesized having the sequence of the generated microRNA.

19. (Currently Amended) A system for identifying a microRNA-recognition element comprising:

an input interface for inputting mRNA sequences, a database of mRNA sequences or a link for connecting to a remote data input interface, data or a database of mRNA sequences;

an input interface for inputting microRNA sequences, a database of microRNA sequences or a link for connecting to a remote data input interface, data or a database of microRNA sequences;

a processor with instructions for comparing mRNA sequences to microRNA sequences to identify a microRNA-recognition element according to the method of ~~any of~~ claims 12-17.

20. (Currently Amended) The system of claims 19 comprising a link for connecting to a database of mRNA sequences.
21. (Currently Amended) The system of claims 19 comprising an input interface for inputting microRNA sequences.
22. (Currently Amended) A computer program embodied on a computer readable medium for implementation on a computer system that for identifying a microRNA-recognition element, the program comprising instructions for performing the steps of the method of ~~any of~~ claims 12-17.
23. (Currently Amended) An recombinant nucleic acid molecule comprising a heterologous coding sequences and one or more MREs identified by the method of ~~any of~~ claims 1-7.
24. (Currently Amended) An isolated nucleic acid molecule comprising one or more MRE sequences identified by the method of ~~any of~~ claims 1-7, said nucleic acid molecule being free of a coding sequence operably linked to regulatory elements.
25. (Currently Amended) A microRNA generated by a method according to ~~any of~~ claims 12-17.
26. (Currently Amended) A method of down regulating expression of an mRNA comprising the steps of selecting a nucleotide sequence of the mRNA, generating a microRNA according to ~~any of~~ claims 12-17, synthesizing an oligonucleotide having the microRNA sequence and contacting the microRNA with a cell expressing the mRNA.